THE HEATS OF WETTING OF SILK FIBROIN AND CELLULOSE BY WATER

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ABSTRACT

The heats of wetting of silk fibroin samples with various initial moisture contents were obtained by means of an adiabatic calorimeter.

From the heat of wetting measurements, and from a water vapour adsorption isotherm, the heat, free energy and entropy of adsorption were calculated.

Two different methods of calculating the surface area of the silk were used and an explanation of the wide divergence of results was offered.

The heats of wetting of cotton linter samples which had undergone various numbers of wetting and drying cycles were also measured. An explanation was made to correlate the heat measurements with the accessibility of cellulose to various reagents.

An attempt to measure the heat of 'wetting' of wool keratin was made.

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INTRODUCTION

The object of this work was to measure the heats of wetting by water of wool and silk. These data for samples with different moisture contents, from zero moisture to saturation, together with data from water vapour adsorption isotherms would permit an evaluation of certain thermodynamic functions for the adsorption process. Heat of wetting measurements by water on specially prepared cellulose samples were also carried out to corroborate other experimental work done by Dr. R.K. Brown and co-workers. All heat of wetting measurements were done at 25°C.

Silk and Wool

Many workers have measured water vapour adsorption isotherms on crystalline proteins by applying the Brunauer-Emmett-Teller (BET) equation (1). It is possible to interpret these isotherms in terms of surface area and structure of the solid adsorbent. Much work has also been done on inert gas adsorption on proteins. The BET plots of these isotherms give different results with regard to surface area -- the water vapour data giving areas 40 to 250 times as large as those obtained from the 'inert' gas isotherms (2). This has been interpreted as meaning that the water vapour adsorption occurs more strongly on specific sites on the molecule, namely the peptide linkages and polar side chains. In other words, the surface area calculated from the BET equation for the water vapour isotherms is much larger because the proteins in general present a large internal surface to the water molecules, not accessible to 'inert' gases such as nitrogen.

By applying thermodynamics to water vapour adsorption isotherms run at 25° and 40°, it is possible to calculate the free energy change of the adsorption process. These calculations have been done by Davis

and McLaren (3) on isotherms run by Bull (4). From this free energy data for the two isotherms it is then possible to calculate the heats of adsorption as a function of volume of water adsorbed. However, as this is an indirect calculation in which only the general trend of the ΔH curve can be ascertained, the use of these data to calculate entropy change gives even more inaccurate results.

For insoluble proteins the wetting process can be considered to be closely related to water vapour adsorption, the main difference being the phase in which the adsorbate exists. For soluble proteins solution and dilution also occur when the protein is mixed with liquid water, so that no close relation exists between wetting with liquid water and water vapour adsorption. The aim of this project was to measure the heats of wetting of typical insoluble proteins, namely wool and silk. These data would give direct values for the heat of adsorption and thus permit a more accurate calculation of entropy changes.

Wool belongs to the class of proteins known as keratins, which are highly insoluble in water. Other examples of keratins are hair, horn, nails, and hoof. Keratins are characterized by a relatively large percentage of sulphur (contained in the amino acids cystine and cysteine) and also a large percentage of aromatic amino acids.

Pure silk is known as fibroin and consists largely of the smallest neutral amino acids: glycine and alanine. Raw silk consists of about 30% by weight of "gum" or sericin, which must be removed by what is called the degumming process.

The only values of heats of wetting by water of wool are those reported by Hedges (5a) and also by MacMillan, Mukherju and Sen (5b), who used a Dewar flask and Beckmann thermometer to determine the heats of wetting. He also reported four values of

the heat of wetting of silk with different initial moisture contents.
His technique was incapable of yielding as accurate results as those
obtained by our method of calorimetry.

Cellulose

The following discussion on experimentally determined facts
on cellulose is given as a background for the heat of wetting measure-
ments on cellulose reported in this thesis.

Cellulose is considered to consist of crystalline areas
which are not readily reactive with various reagents, and less crys-
talline areas where the hydroxyl groups are comparatively easily
reached by these reagents. The percentage of hydroxyl groups which
is available for reaction is considered an indication of the accessibility
of cellulose.

In speaking of degree of accessibility one must specify what
reagent is being used. An ordinary sample of cellulose can be methylated
(using thallous ethylate in ether and then methyl iodide) to the extent
of about 2%. In other words, 2% of the total number of hydroxyls are
reacted. If the same sample were nitrated using nitric acid for one
half hour (the commercial method of nitrating) a nitrogen analysis of
the resulting cellulose nitrate would indicate approximately 12%
nitrogen.

If a sample of cellulose were swollen with cold 10% sodium
hydroxide which was then removed by the method of solvent exchange
(neutralizing with acetic acid, then washing with water, methyl
alcohol, dry methyl alcohol and dry benzene), the sample would remain
in a highly swollen condition, that is, there would be a relatively
small number of hydroxyls not available for reaction. Methylation
of this sample by the above method gives an accessibility of about

35%. Nitration of this sample would give a value of about 12.6% nitrogen. The reason for the marked increase in accessibility to the methylating reagent, as compared to the relatively small increase in accessibility to nitric acid, is that the methylating reagent is non-swelling and therefore, does not change the cellulose structure, whereas the nitric acid itself will swell the cellulose so that only a small relative difference is obtained.

If the swollen cellulose is wetted once with water and then dried, a change known as the "zippering effect" occurs. The water reacts with the cellulose hydroxyl groups to form hydrogen bonds, then as the water is withdrawn, adjacent chains of the cellulose are drawn closer together and hydrogen bonds are formed between the hydroxyl groups of these adjacent chains. This is in effect decreasing the accessibility of the sample by increasing the percentage of crystalline cellulose.

Methylation of a swollen sample which has undergone one wetting-drying cycle as outlined above gives about 2% accessibility to the methylating reagent whereas nitration gives a high value of about 12.4% nitrogen.

The purpose of our work was to determine the effect of different numbers of wetting and drying cycles on the accessibility of the cellulose to water vapour. Results on methylation and nitration of such samples indicate the same general trend. The cellulose with one cycle was slightly less reactive to both reagents than the sample which had undergone no cycles; then, as the number of cycles increased, the reactivity of the samples gradually increased also. It must be emphasized that although these changes in accessibility are very small, they are of great significance. In the commercial preparation of

cellulose nitrate the differences in percentage nitrogen obtained between batches which are soluble in the solvent used and batches which are insoluble and must be discarded are of the same order of magnitude.

If samples of cellulose which have undergone consecutive numbers of wetting and drying cycles are placed in an atmosphere of constant relative humidity, an increase in accessibility to water vapour is noted in the sample which has undergone one cycle as compared to the sample with no cycles. Then as the number of cycles is increased a gradual decrease in accessibility is noted. This is the reverse of the effect noted in the methylation and nitration reactions and at first appears to be anomalous (6).

It was decided to carry out heat of wetting measurements on the above cellulose samples in order to determine whether there is any relationship between heats of wetting and the number of wetting-drying cycles that the cellulose has undergone.

The first part of the problem was to construct a calorimeter to carry out these measurements. It was decided to use a rotating adiabatic calorimeter based on the design of Lipsett, Johnson, and Maass (7). Adiabatic control gives the most accurate results for this type of work. Because a calorimeter with a small heat capacity is desired so that the temperature change obtained is as large as practicable, it is very difficult to have a stationary calorimeter with an efficient stirring mechanism. The alternative is to have the calorimeter rotating to insure intimate mixing of the sample with water. This complicates the temperature readings, however, for it is virtually impossible to seal any kind of a thermometer or thermopile into a rotating calorimeter. However, differences between bath and calorimeter temperatures can be determined by means of a radiation thermopile. The exact description of the original calorimeter can be found from the above reference and all auxiliary equipment is described in a previous article by the same authors (8). Therefore, only a brief description of apparatus including a few small changes from the original will be made here. The radiation thermopile has a sensitivity of 1×10^{-4}°C per mm. galvanometer scale deflection. Initial and final calorimeter temperatures are obtained indirectly by measuring the bath temperature with a platinum resistance thermometer and a Meuller bridge. The bridge can be read to 10^{-4} ohms which is equivalent to 10^{-3}°C. However, by maintaining adiabatic control by means of a fine stream of hot water added to the bath, and observing the galvanometer deflections for each Meuller bridge reading, the calorimeter's temperature can be calculated to four decimal places.

The slight changes made in the design of the apparatus are as follows:

No support was used to hold the thermopile in place other than the shellac placed on the lower junctions. The thin glass supports used on the original apparatus were found not only to be unnecessary, but an added difficulty to the construction of the thermopile.

A stainless steel plug was used to seal the opening through which water was pipetted into the calorimeter. Vaseline was used to ensure a water-tight seal here as well as on the end flange of the calorimeter.

All switches were of the mercury commutator type, it being found that they were the simplest type with virtually no contact potential.

The flange on the copper jacket which contained the calorimeter was held secure using nuts and bolts instead of clamps as on the original apparatus. The bolts were secured to the lower flange and passed through correspondingly placed holes in the upper flange. Specially designed nuts were used, being nearly $1\frac{1}{2}$" in length and having handles on them so they could be easily screwed on by hand, without hindrance from the upper part of the copper jacket. A gasket of "Garlock" gasket material was used to keep the flange water-tight.

The water bath was fitted with a cover to shelter it from any drafts in the laboratory. This was critical because any change in the rate of evaporation made the maintenance of adiabatic control difficult. However, part of the early difficulties experienced in operating the calorimeter, which were attributed to this cause, are now known to have been due to a very slight evaporation of water from

the calorimeter to the air space of the copper jacket, because of imperfect machining of the end flange of the calorimeter.

A desiccator was specially designed to contain the oven-dried samples till they were cooled to room temperature. It was constructed from a metal container 9" in diameter and 5" in depth. The top of the container was replaced with a circle of glass having a hole 4" in diameter which could be covered with an ordinary small desiccator lid to give an air-tight seal. This glass was sealed on using DeKhotinsky cement. Phosphorus pentoxide was contained in small trays placed inside the desiccator. The relatively small opening kept the transfer of outside air into the desiccator at a minimum. Originally, a rubber glove was sealed into the side of the desiccator, so that upon cooling, the dried sample could be covered before the desiccator was opened. This was found to be unnecessary, if the precaution was taken to cover the sample immediately after the desiccator was opened.

Samples whose heats of wetting are to be determined are placed in the inner box which is covered with a vaselined lid to give an air-tight seal. This is held in a fixed position inside the calorimeter. A weighed amount of water is added to the calorimeter which is then placed inside the copper jacket. The bath is then filled and the temperature of bath and calorimeter brought to $25 \pm 0.1°C$. After obtaining consecutive identical readings of the initial temperature, rotation of the calorimeter is begun, adiabatic control being maintained as closely as possible when the wetting reaction is occurring. Considerable difficulty was experienced at first in obtaining a lid for the inner box which was machined true enough to give an air-tight seal, heavy enough to fall off to permit mixing of the sample with

water when rotation was begun, and yet of the correct dimensions to
be caught by the arresting "hooks" designed to prevent its free
movement inside the calorimeter during rotation. After a fixed period
of rotation, the rotation was ceased and the final temperature obtained
in the same manner as the initial temperature. The heat capacity of
the calorimeter, which was almost entirely constructed of sterling
silver, was 13.09 calories plus a correction for the evaporation of
water vapour into the inner box when rotation was commenced. A
correction for the heat of rotation was also made. Knowing the sample
weight and the heat capacities of the calorimeter, water, and the
sample, as well as the temperature rise, it is a simple matter to
calculate the heat of wetting in calories per gram of sample. A
sample calculation is enclosed.

<u>Run #11 on Silk Fibroin</u>   January 2, 1952.

1. Wt. of sample box	29.0439 g.	Wt. of calorimeter +	
2. Wt. of greased lid	34.1370	support	219.012 g.
Wt. of 1. + 2.	63.1809	Wt. of above + H_2O	268.767
Wt. of 1. + 2. +		Weight of water	49.755
dried sample	65.5082		
Dry sample weight	2.3273		

<u>Resistance of Pt Thermohm</u> (in ohms)   <u>Time</u> (in mins.)

28.03680		0
28.03685		2
28.03680		4
28.03680		6
28.03672		8
28.03675	Mean 28.03679	10
Start of rotation		$10\frac{1}{2}$
End of rotation		$30\frac{1}{2}$
28.09485		32
28.09486		34
28.09483	Mean 28.09485	35
Change in resistance	0.05806 ohms	
Correction for ht. of		
rotation	0.00050	
Net resistance change	0.05756 ohms	

Net temperature change $\dfrac{0.05756}{0.10080}$ = 0.5714°C.

Heat capacity of calorimeter = 13.59
Heat capacity of water   = 49.68
Heat capacity of silk   =   0.78 (2.33 x 0.33)
                                ─────
                                64.05

Heat of wetting = $\dfrac{64.05 \times 0.5714}{2.327}$ = 15.73 cal./gm.

        All the required specific heats were obtained from the International Critical Tables. The weight of water was always corrected to the weight in vacuo.

        An experimental check on the calculated heat capacity was obtained by the method used on the original apparatus (7). This consisted essentially of measuring the heat of solution of sodium chloride, using the same ratios of salt to water for two determinations but using different amounts of reactants. As the concentration of salt is the same in each case, the heat of solution will be the same. However, the temperature drop occurring will be different for the two different amounts of reactants. The only unknown is the heat capacity of the calorimeter. It can be solved for by using two simultaneous equations.

        A water vapour adsorption apparatus identical with that of Wiig and Juhola (9) was used to obtain the isotherm for silk fibroin.

Wool and Silk

The wool was prepared by Dr. Larose of the National Research
Council at Ottawa. He purified the wool by extracting with alcohol
and ether and equilibrating to a pH of 5. The silk was obtained in
the raw state from the Belding-Corticelli plant at St. Jean, Quebec.
It was degummed by the method of Sookne and Harris (10).

It was originally decided to dry all samples in a manner
similar to that used by Hedges (4), so that comparable data would be
obtained. The procedure used was as follows. A stream of air from
a compressed air line was passed through some absorbent cotton, then
through two scrubbers of concentrated sulfuric acid, then a tower
of sodium hydroxide pellets, then through two towers containing
dehydrite (magnesium perchlorate), then through a copper coil con-
tained inside an oven maintained at $105 \pm 1°$. This preheated the
air before it entered the desiccator containing the sample to be
dried. The drying process lasted for two hours after which the
sample was placed in the previously described desiccator (see page 8)
over phosphorus pentoxide for thirty minutes to cool. It was then
weighed to obtain its dry weight.

If the sample were prepared to determine the heat of wetting
with no initial moisture content, the container used for drying the
sample was the inner box of the calorimeter. As soon as the dried
weight was obtained (by difference) the inner box, covered by the
greased lid, was placed in the calorimeter and the heat measurement
carried out.

If, however, the sample were being prepared to obtain a
heat of wetting for a certain initial moisture content, the container

used for drying was a weighing bottle. As soon as the dried weight was obtained, the sample contained in the weighing bottle was placed over a sulfuric acid solution of the desired concentration to give the required initial moisture content. After being left for at least three days, it was transferred as rapidly as possible to the inner box, covered with the weighed greased lid, and reweighed to determine the percentage moisture content. Then the heat measurement was carried out.

However, because Bull's data were obtained on vacuum dried samples, and because the water vapour adsorption apparatus used was also high vacuum apparatus, it was decided to determine if any difference would be obtained for samples which were vacuum dried. Three samples which were dried by the method previously described were vacuum dried for two hours using an Aberhalden vacuum drying apparatus, commonly called a drying pistol, containing boiling water to heat the samples. After the samples were allowed to cool, dried air was admitted into the pistol through the series of driers mentioned in the previous procedure. The sample was then withdrawn from the pistol, covered with the weighed greased lid and reweighed to obtain the dry weight on this basis. These samples were found to be slightly drier than samples prepared according to Hedges' method and gave slightly higher values for the heat of wetting. All the previous data were corrected to allow for this difference.

Cellulose

To check the experimental procedure used, some "standard" cellulose as defined by Argue and Maass (11) was prepared and its heat of wetting was determined.

The heat of wetting measurements carried out for Dr. Brown

were on cotton linter samples. These were obtained from the Hercules
Powder Company and were dewaxed with a benzene-alcohol mixture (6).
Samples of this cellulose were then subjected to wetting and drying
cycles. The heat measurements carried out were on samples which
had undergone from 0 to 19 of these cycles.

Each sample on which a heat of wetting measurement was carried
out, was removed from the desiccator containing phosphorus pentoxide,
which was used for storing the samples, and placed in the inner box
of the calorimeter. This procedure enabled the sample to pick up
moisture from the air of the laboratory, so each sample was oven dried
for two hours, using the same procedure as was used for the silk.
The uniform drying gave samples on which comparable heat of wetting
data could be obtained.

Calibration of Calorimeter

The results of 14 determinations of the heat of solution
of sodium chloride (seven with one quantity of reactants and seven
with a different quantity, the ratio of salt to water being the same
in both cases) gave an experimentally determined heat capacity within
0.08 calories of the calculated value. This is considered to be a
slight error, because although it amounts to about six parts per
thousand for the metal calorimeter itself (calculated heat capacity
13.09 calories), it only amounts to about 1 part per thousand for the
total heat capacity of the calorimeter (including 50 ml. of accurately
weighed water, which was the quantity used for all heat of wetting
measurements).

Silk

The heat of wetting values obtained for silk are given
in Table I and plotted in Figure 1, together with the four values
reported by Hedges (5). All the results in Table I were obtained on
the same basis of drying: two hours in a stream of dried air at
105°C.

It is difficult to estimate the accuracy of the results
obtained, for though a uniform technique may give a high degree of
precision, the results obtained would probably differ slightly for
different workers. The method of handling the samples is extremely
critical because of their great affinity for moisture. (It was found
that the samples had to be cooled over phosphorus pentoxide the same
length of time after removal from the drying oven in order to get
reproducible results.) Some comparison of the degree of precision
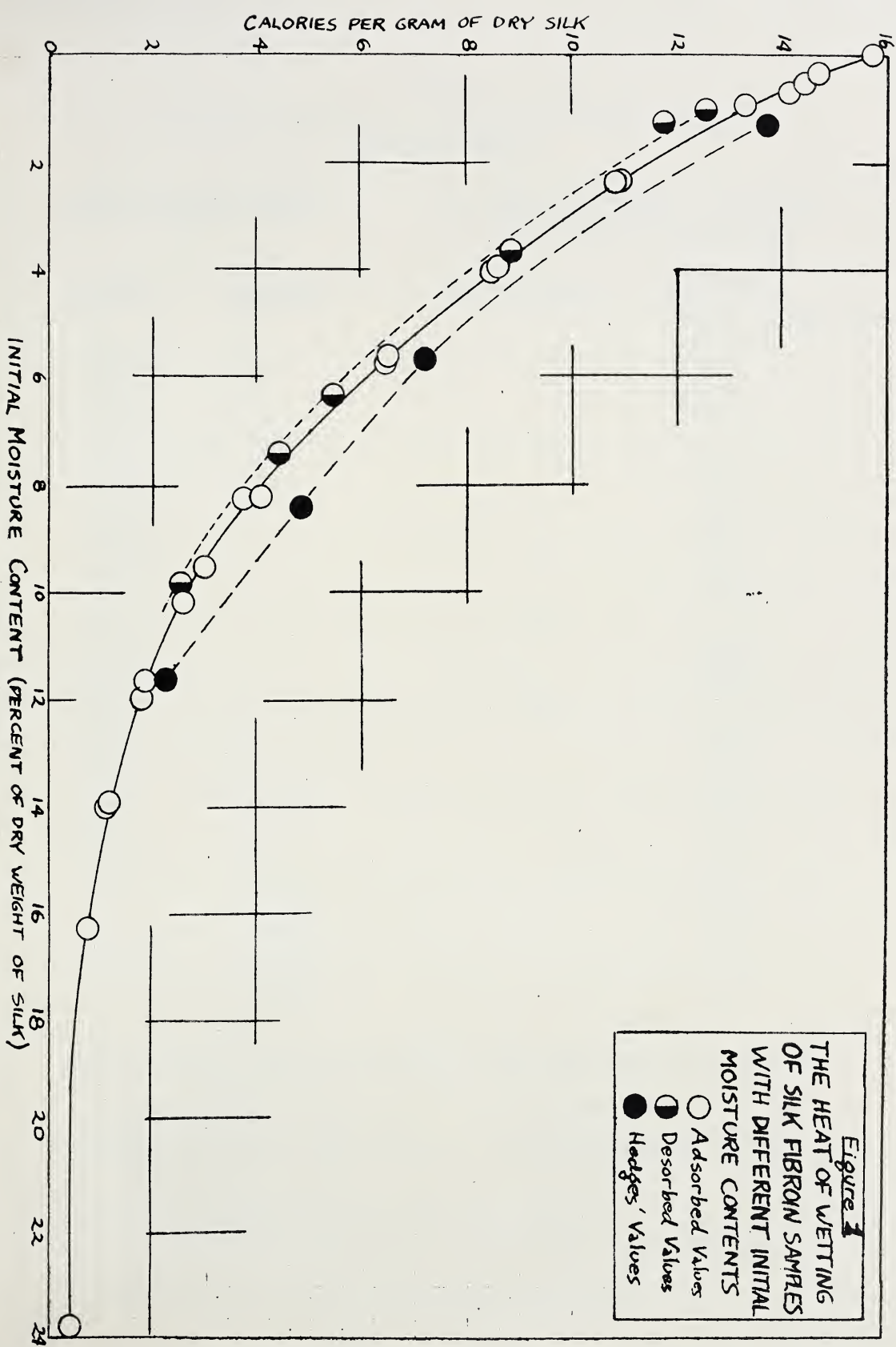of our method of calorimetry with that of Hedges may be seen from

Figure 1
THE HEAT OF WETTING
OF SILK FIBROIN SAMPLES
WITH DIFFERENT INITIAL
MOISTURE CONTENTS

○ Adsorbed Values
◐ Desorbed Values
● Hodges' Values

TABLE I

HEAT OF WETTING OF SILK FIBROIN SAMPLES WITH DIFFERENT INITIAL MOISTURE CONTENTS

Initial Moisture Content (% Dry Weight of Silk)		Heat of Wetting (Cal./gm. of Dry Silk in increasing water content to saturation).	
Adsorbed	Desorbed	Hedges' Values	Our Values
0			15.67, 15.73, 15.72
0.34			14.64
0.51			14.40
0.66			14.09
0.91			13.24
1.3		13.0	
2.32			10.92
2.36			10.78
3.94			8.51
4.03			8.37
5.6(0)		7.1	6.42
5.70			6.39
8.18			4.03
8.2		4.8	
8.25			3.70
9.53			2.98
10.19			2.51
11.6		2.1	
11.67			1.83
11.91			1.68
13.99			1.13
14.00			1.03
16.2			0.69
23.7			0.34
25.0			0.19
	1.04		12.47
	1.25		11.72
	3.62		8.83
	5.44		6.31
	7.39		4.36
	9.78		2.48

the following. The mean deviation of our three values for the heat of wetting values of dry silk is ± 0.03 cal./gm., while the mean deviation on Hedges' four values for the heat of "wetting" of dry wool is ± 0.4 cal./gm. (Mean value 24.1 cal./gm.)

The theoretical limit on the accuracy of the temperature readings is ± 1 x 10⁻⁴°C. In practice it was found to be about

± 4 x 10⁻⁴°C. Thus for the initial and final readings necessary for
each temperature rise, the accuracy would be about 1.5 parts per
thousand for the largest temperature rises obtained (0.55°C), and
about 17 parts per hundred for the smallest temperature rise
(0.0048°C).

It can be seen from Figure 1 that the plot of the heats
of wetting of the "desorbed" samples (samples which are saturated
with water in an atmosphere of 100% relative humidity and are then
partially dried over sulfuric acid solutions) falls slightly below the
plot of the heats of wetting of the "adsorbed" samples. This corre-
sponds to the hysteresis effect obtained in the water vapour adsorption-
desorption isotherm (see Table III and Figure 2) but is the reverse
of the results obtained by Argue (11) on cellulose. This leads to
the conclusion that the effects occurring in the adsorption and
desorption of water vapour on and from cellulose are not analogous
to those occurring in silk.

From the value obtained for the heat of wetting or immersion
of a sample which is saturated with water vapour (the final "adsorbed"
value listed in Table I) it is possible to calculate a value for the
surface area of the silk according to Harkins' and Jura's "absolute"
method (12). This consists essentially of calculating the area of the
surface film adsorbed on the silk from the heat liberated when the
sample is immersed in water and the surface film energy is liberated.
The value obtained was 7 square meters per gram of silk. (The error
by our method of calorimetry is estimated as about 20%). However,
swelling effects are also believed to occur when the silk sample is
immersed in liquid water (3) so that the above surface area value is
further in error. If however, another method of obtaining the surface

area is used (see the discussion following the water vapour adsorption data) a method of obtaining an estimate of the heat of swelling is available.

As explained under the heading of "Preparation of Materials", it was decided to vacuum-dry some silk samples to determine whether drier samples could be obtained. This was found to be the case; three determinations showed that the oven dried samples contained 0.20% moisture on the vacuum dried basis. The value of 15.93 cal./gm. was obtained for the heat of wetting of the vacuum dried samples. All previous data were corrected to this basis of drying so that they would correspond to water vapour adsorption isotherms. The corrected data were replotted as calories per 100 grams of dry silk against moles of water per 100 grams of dry silk. The values reported in columns 1 and 2 of Table II were read from this graph. This procedure simplified the subsequent calculations and added little to the error involved. All values reported are for the "adsorbed" samples.

The samples which have an initial moisture content give lower values for the heats of wetting because of the water vapour already adsorbed on them. Thus the differences between the mean heat of wetting of the dry samples and the heats of wetting of the samples with the initial moisture contents are taken as the heats of adsorption of the number of moles of water given in column 1 on 100 grams of dry silk (recorded in column 3).

The values given in the fifth column (obtained by multiplying the data of columns 3 and 4) represent the heats of adsorption of one mole of water on an amount of dry silk sufficient to give the concentration listed in the first column.

The final column in Table II represents the partial molal

TABLE II

CALCULATION OF HEAT OF ADSORPTION DATA OF WATER VAPOUR ON SILK FIBROIN

n Initial Moisture Content (moles of water/100gm. silk)	Heat of Wetting (Cal./100gm. dry silk in increasing water content to saturation.	ΔH Heat of Adsorption (Cal./100 gm. dry silk).	$\frac{1}{n}$ No. of 100gm. silk containing 1 mole of water at conc. in col. 1.	$\frac{\Delta H}{n}$	$\frac{\partial(\Delta H)}{\partial n}$
0	1595	0	∞		4700
0.05	1390	205	20.00	4010	3750
0.10	1210	385	10.00	3850	3270
0.15	1055	540	6.67	3600	2960
0.20	920	675	5.00	3370	2560
0.25	795	800	4.00	3200	2350
0.30	685	910	3.33	3030	2140
0.35	590	1005	2.86	2880	1940
0.40	500	1095	2.50	2740	1730
0.45	410	1185	2.22	2630	1530
0.50	340	1255	2.00	2510	1270
0.55	280	1315	1.82	2380	1070
0.60	230	1365	1.67	2280	890
0.65	190	1405	1.54	2160	720
0.70	155	1440	1.43	2060	595
0.75	130	1465	1.33	1950	500
0.80	110	1485	1.25	1860	415
0.85	90	1505	1.18	1780	305
0.90	75	1520	1.11	1690	255
1.00	60	1535	1.00	1540	120
1.20	35	1560	0.83	1300	35
1.40	25	1570	0.71	1120	0

heats of adsorption (plotted in Figure 4). The values are obtained

by evaluating graphically the tangents to the curve given by the data

in columns 1 and 3. The partial molal heat of adsorption is the

heat of adsorption of one mole of water on an infinite amount of silk

(great enough that the moisture content of the silk remains unchanged.)

The results for the water vapour adsorption-desorption

isotherm are included in Table III and plotted in Figure 2. Bull (4)

and other workers (13) have obtained water vapour adsorption isotherms

on silk -- the chief purpose of repeating the work was to obtain the

isotherm from the same batch of silk that was used for the heat of

wetting measurements, so that comparable data would be ensured.

TABLE III

THE WATER VAPOUR ADSORPTION-DESORPTION ISOTHERM (25°C) FOR SILK FIBROIN
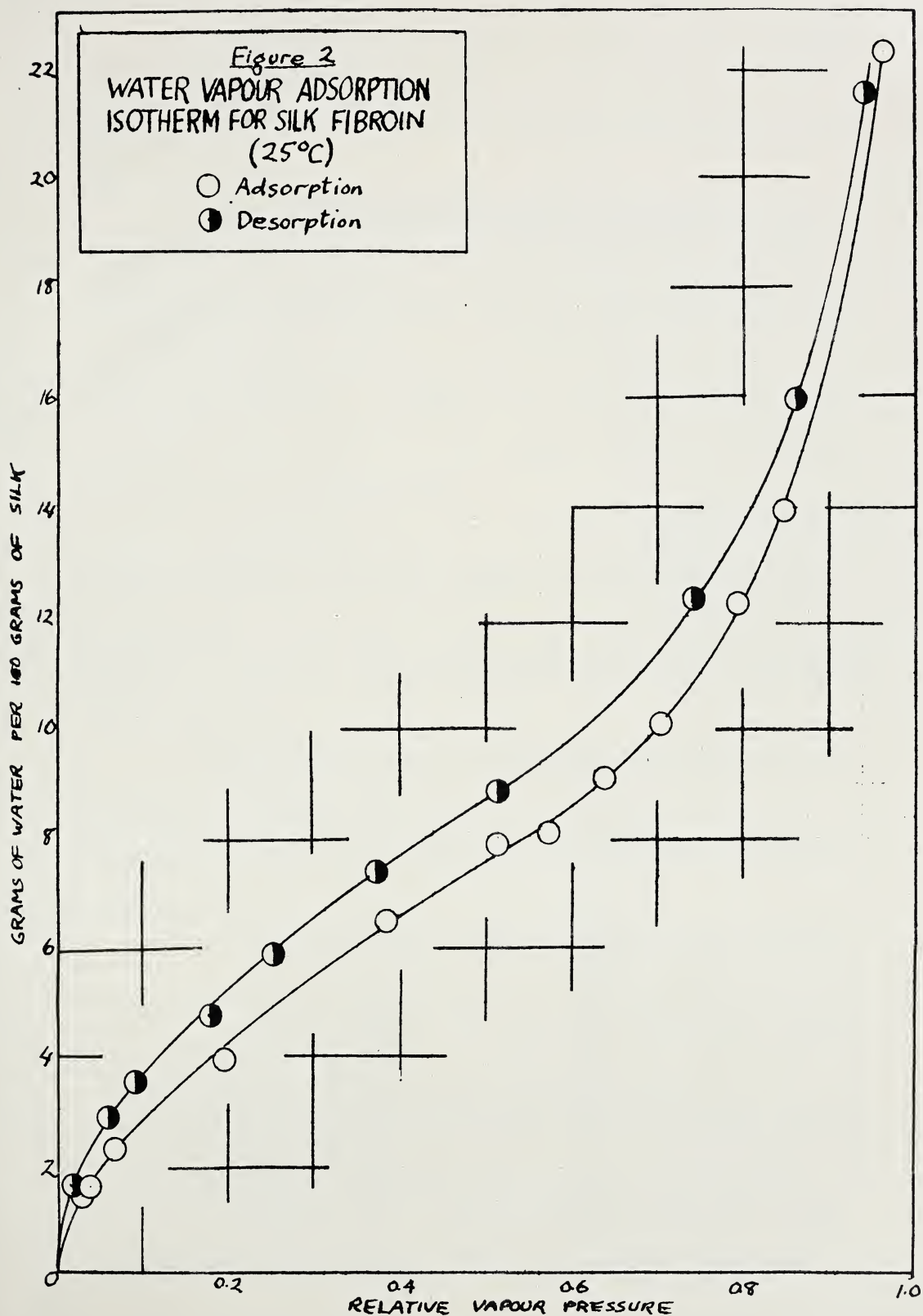AND CALCULATION OF FREE ENERGY OF THE ADSORPTION REACTION

Moles water/100 gm. silk		Relative Vapour Pressure $\frac{P}{P_o}$	Differential Free Energy of Adsorption (Cal./mole of water) $\frac{dF}{dn} = RT \ln \frac{P}{P_o}$
Adsorption	Desorption		
0.0784		0.0262	2160
0.0885		0.0380	1940
0.126		0.0668	1600
0.218		0.194	970
0.358		0.384	570
0.436		0.513	395
0.449		0.574	330
0.506		0.638	240
0.559		0.703	210
0.679		0.794	135
0.775		0.848	95
1.24		0.962	20
	1.20	0.94	
	0.887	0.86	
	0.685	0.74	
	0.491	0.51	
	0.410	0.37	
	0.324	0.24	
	0.263	0.18	
	0.196	0.09	
	0.158	0.06	
	0.089	0.02	

The plot of the isotherm in Figure 2 indicates that a smooth sigmoid shaped curve is obtained. It might be noted that the agreement with Bull's isotherm (which was obtained by the desiccator method) is good, the two curves coinciding almost exactly. The chief difference appears to be that our isotherm exhibits slightly more hysteresis. Whether this is caused by the effect of the dibutyl phthalate used in the manometers of the apparatus, or because insufficient time was allowed to attain equilibrium remains to be determined. It was found that three to four hours were required to attain steady readings at each new vapour pressure; it might be worthwhile to double this time and see if any appreciable drift in pressure is obtained.

Figure 2
WATER VAPOUR ADSORPTION
ISOTHERM FOR SILK FIBROIN
(25°C)
○ Adsorption
◑ Desorption

GRAMS OF WATER PER 100 GRAMS OF SILK

RELATIVE VAPOUR PRESSURE

The BET plot of the adsorption isotherm is given in Figure 3a. It gives a calculated monomolecular surface area of 157 square meters per gram of silk, compared to Bull's BET value of 144 m.^{2} per gm.

A plot of the adsorption isotherm data according to the Harkins-Jura equation (14) yielded a good straight line plot for relative vapour pressures greater than 0.5. The surface area of the silk calculated from the slope of the line (see Figure 3b) is 1.63 square meters per gram. It might be noted that no attempt to apply the Harkins-Jura equation to a water vapour adsorption isotherm on silk was found in the literature.

Using the above value of 1.6 m.^{2}/gm. together with the amount of heat liberated by immersing a sample saturated with water vapour (see Table I), a value for the heat of swelling of silk of 0.015 cal./gm. is obtained. The swelling effect is thus seen to be practically negligible.

The two surface areas calculated from the plots shown in Figure 3 are not inconsistent with the present-day concept of the adsorption process, though they differ from each other by a factor of nearly 100.

Recent statistical treatments of the adsorption isotherm (15,16) have shown that the BET type of theory is based on the adsorption on localized sights, rather than the formation of a mobile adsorbed monolayer. Thus one would expect the BET plot of the water vapour adsorption data to give a straight line plot up to a relative vapour pressure of 0.5 (about as high as it goes on any type of adsorption process) for it is generally accepted that water vapour adsorption on proteins occurs first on the peptide linkages and
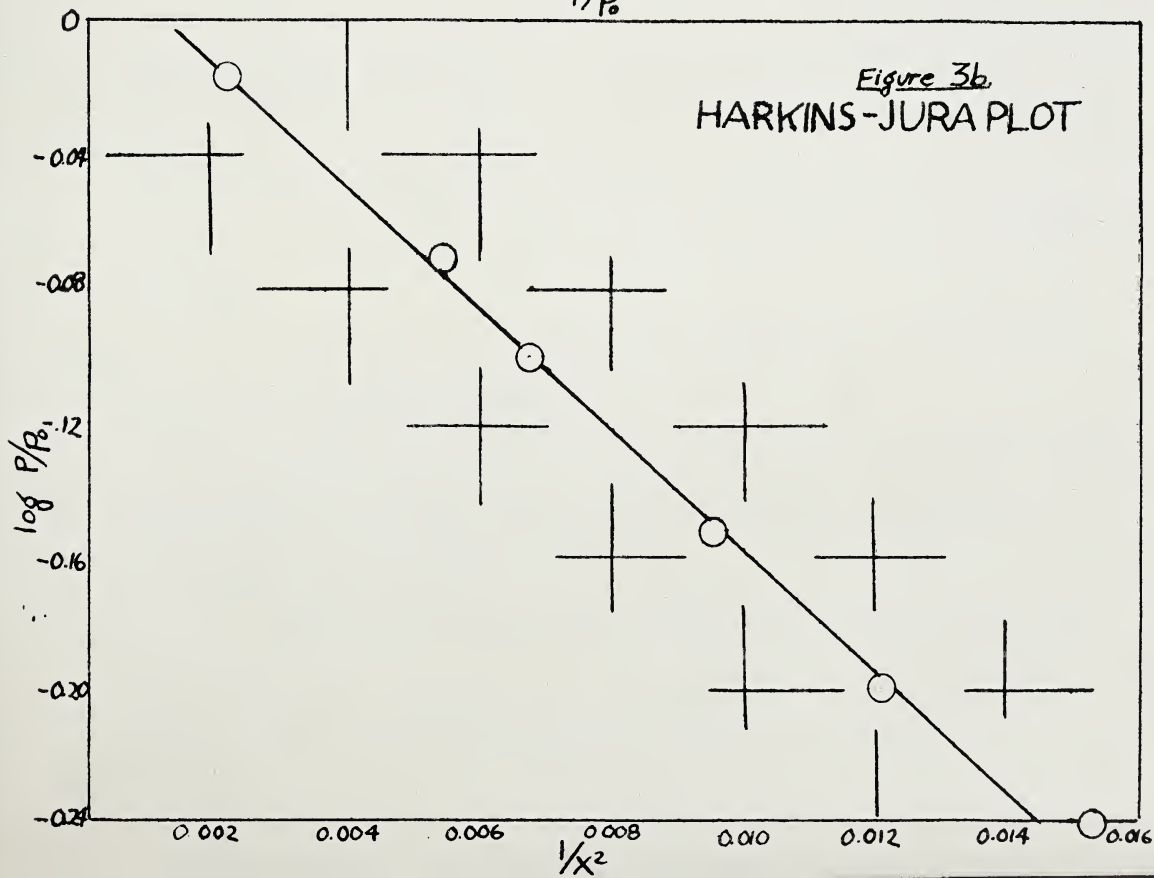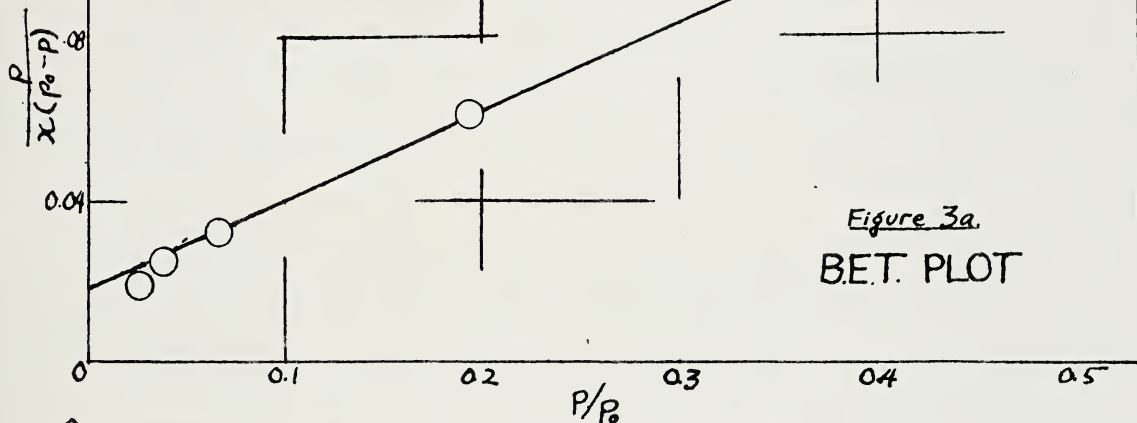
Figure 3

PLOTS OF WATER VAPOUR
ADSORPTION DATA TO
DETERMINE THE SURFACE
AREA OF SILK FIBROIN

p = vapour pressure
P_0 = saturation vap. press.
x = gms. H_2O per 100 gms. silk

Figure 3a.
B.E.T. PLOT

Figure 3b.
HARKINS-JURA PLOT

polar side chains (17). (That the polar side chains alone are respon-sible for the formation of the adsorbed monolayer, with the peptide linkages having virtually no effect, was postulated by Pauling (18) but this interpretation has been questioned (19)).
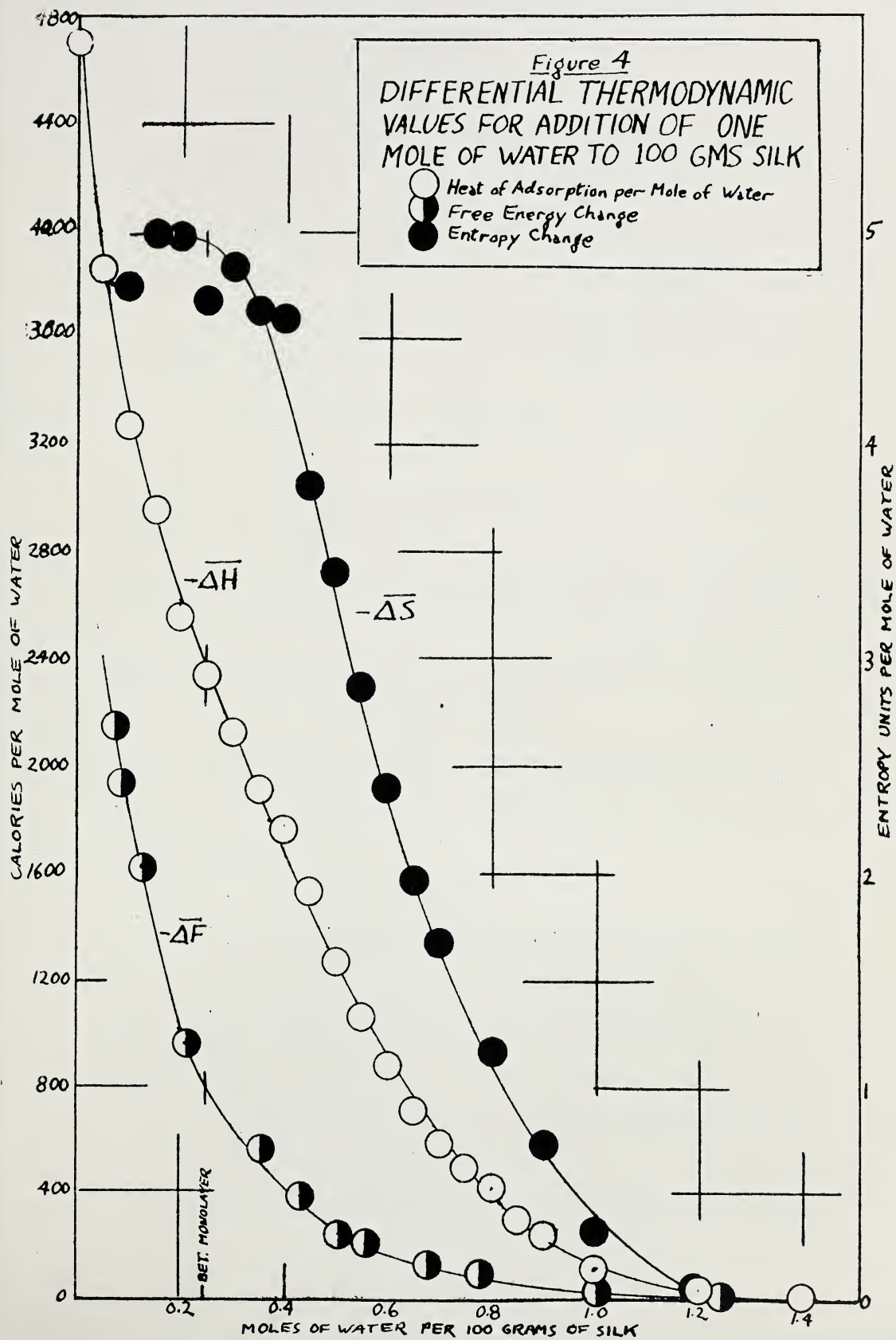
On the other hand, Harkins' and Jura's interpretation of the adsorption isotherm is based on the assumption that a condensed film is formed. This may be the case for silk above 0.5 relative vapour pressure. Davis and McLaren (3) postulated a spectrum of sorption taking place on silk, with chemisorption being the predominant effect at lower vapour pressures, but still occurring right up to saturation vapour pressure and even when a saturated sample is wetted with liquid water. This could well be the case at lower vapour pressures but may be a negligible effect above 0.5 relative vapour pressure, as shown by the straight line plot in Figure 3b.

The large difference between the two calculated surface areas can thus be accounted for on the basis of adsorption at low vapour pressures on specific sites of a large "internal" surface of the silk; for high relative vapour pressures, the internal surface is saturated, so that the only adsorption taking place is of the condensed film type.

The plot of the free energy data given in the final column of Table III is given in Figure 4.

Figures 4 and 5 represent the differential and integral values, respectively, of the heat, free energy and entropy of adsorption of water vapour on silk fibroin.

From the differential heat values given in Table II and the differential free energy values, evaluated in Table III, the differ-ential entropy change is calculated by means of the formula

Figure 4

DIFFERENTIAL THERMODYNAMIC VALUES FOR ADDITION OF ONE MOLE OF WATER TO 100 GMS SILK

○ Heat of Adsorption per Mole of Water
◐ Free Energy Change
● Entropy Change

$$\overline{\Delta S} = \frac{\overline{\Delta H} - \overline{\Delta F}}{T}$$

where T is the absolute temperature.

The high differential entropy values for the monomolecular layer (shown in Figure 4), though not as great as found by Davis and McLaren (3), are still comparable to the entropy of freezing of water (20) and lead to the conclusion that the water is probably chemisorbed.

No increase of the differential heat and entropy values was found as saturation was approached in contrast to the results of Bull (4). Bull interpreted the increase as a species of heat of solution, stating, "In the case of the insoluble proteins, silk and wool, the heat of solution arises from the freeing of segments of the peptide chain from the restriction in motion which was present in the dry state." The present results do not require such an interpretation.

The integral heat values from column 3 of Table II and the integral free energy values, calculated by means of the equation
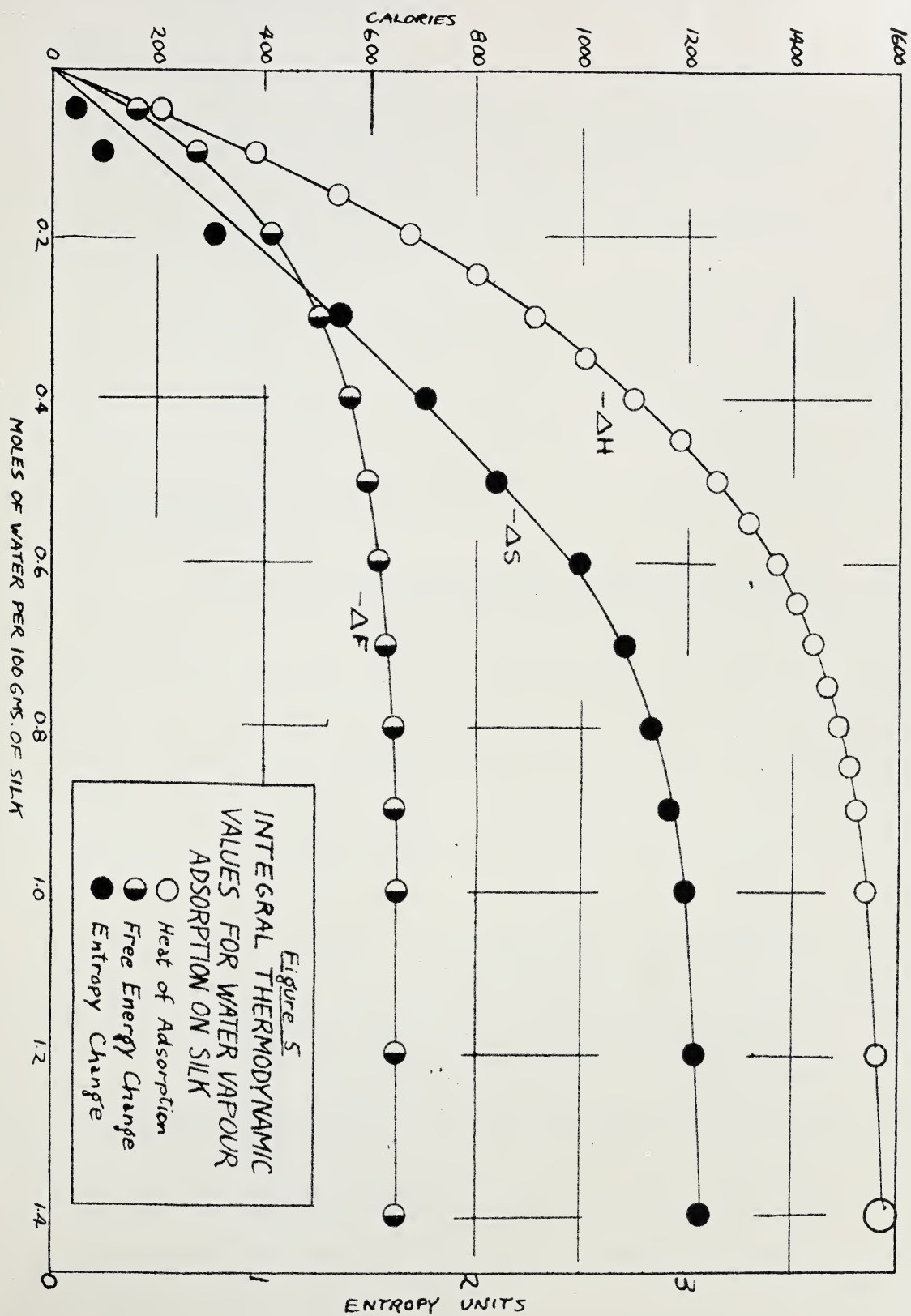
$$\Delta F = RT \int^{n} \ln x \, dn$$

where n is the number of moles of water adsorbed per 100 gm. of silk and x is the relative vapour pressure, lead to the integral entropy change:

$$\Delta S = \frac{\Delta H - \Delta F}{T}$$

Davis' and McLaren's integral ΔS curve increases in an almost linear fashion, contrary to the plot shown in Figure 5. One would expect a rapid increase in the initial entropy values if chemisorption is the predominant initial process.

The above free energy and entropy functions were taken from a paper by Dole and McLaren (21) which contains a comprehensive discussion of the thermodynamics of water adsorption on proteins.

Figure 5

INTEGRAL THERMODYNAMIC
VALUES FOR WATER VAPOUR
ADSORPTION ON SILK

○ Heat of Adsorption
◐ Free Energy Change
● Entropy Change

Wool

No results were obtained on the heat of "wetting" of wool. It was found that even after several hours had elapsed a temperature rise was still occurring, so that the method of calorimetry used to measure the heat of wetting of silk fibroin may be impractical for comparable heat measurements on keratin.

Pure keratin is highly resistant to wetting by water (22), that is an advancing contact angle of greater than 90° is observed for water on a keratin surface. Thus, little or no "wetting" occurs when keratin is immersed in liquid water; the heat evolved is probably largely caused by the adsorption of the water on specific sites on the keratin molecule (16).

Hedges' (5) method of calorimetry consisted of immersing a wool sample in a Dewar flask containing water, and using an up-and-down motion on a stirrer, to cause intimate mixing. Unfortunately, he gives no indication of the length of time required to attain equilibrium. However, it was probably much less than required by our method of calorimetry -- where the water-repellant surface of the wool contained in the inner box prevented any intimate mixing with the water of the calorimeter. The use of a smaller sample to permit greater access to the water gave no improvement. Even if reduction of the sample size had increased the rate of attainment of equilibrium, the theoretical accuracy of the method obviously would be reduced.

Because of the inhibiting effect of air on the adsorption of water vapour by proteins (23), equilibrium could be attained much more rapidly using a vacuum calorimeter. Also because large temperature increases occur when water vapour is adsorbed on keratin (23) a type of calorimeter that ensures intimate mixing with water to prevent local heat effects should be used.

Cellulose

The mean heat of wetting obtained from five samples of standard cellulose was 10.15 calories/gm. This compares with the value of 10.16 cal. obtained by Argue and Maass (11).
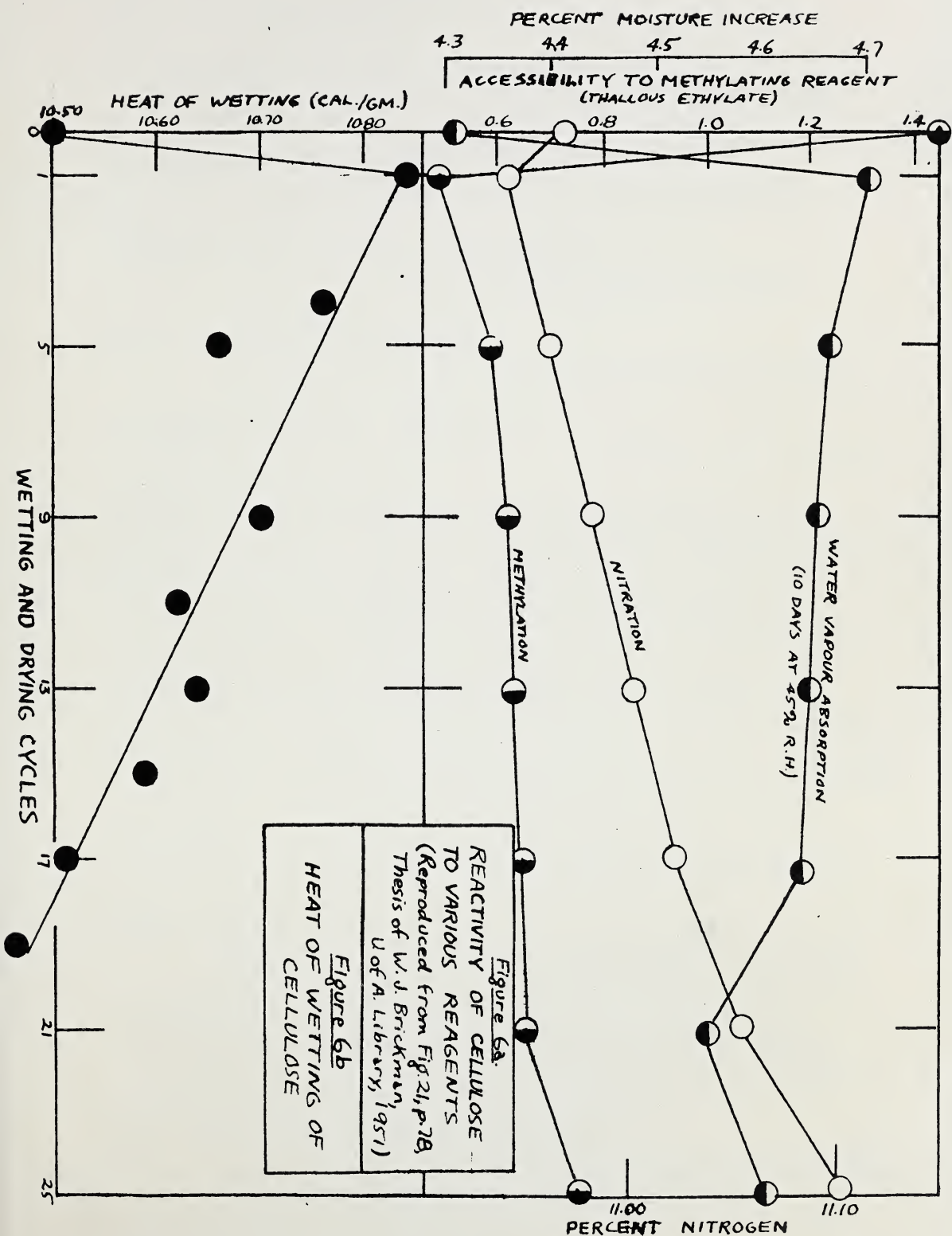
The results obtained for the heat of wetting of cotton linter samples, which had undergone various numbers of wetting and drying cycles, are included in Table IV and plotted in Figure 6. Also shown in Figure 3 are the data for methylation, nitration and water vapour adsorption obtained by Brickman (6).

TABLE IV

VALUES OBTAINED FOR THE HEAT OF WETTING OF COTTON LINTER SAMPLES
WHICH HAD UNDERGONE VARIOUS NUMBERS OF WETTING AND DRYING CYCLES

Run No.	No. of Wettings	Heat of Wetting (calories/gram)	Mean Heat of Wetting
1	0	10.44	
6		10.50	
11		10.57	10.50
10	1	10.84	
12		10.83	10.84
13	3	10.76	10.76
4	5	10.67	
8		10.64	10.66
14	9	10.70	10.70
18	11	10.62	10.62
2	13	10.55	
5		10.72	
9		10.66	10.64
15	15	10.62	10.62
16	17	10.51	10.51
3	19	10.50	
7		10.39	10.45

It can be seen from the plot of the above data that the shape of the heat of wetting curve does not coincide with the curves obtained for methylated and nitrated samples. It does coincide with the plot of water vapour adsorption against the number of wetting and drying cycles obtained by Brickman.

Figure 6a.
REACTIVITY OF CELLULOSE
TO VARIOUS REAGENTS
(Reproduced from Fig.21, p.78,
Thesis of W.J.Brickman,
U.of A.Library,1951)

Figure 6b
HEAT OF WETTING OF
CELLULOSE

The results for water vapour adsorption were obtained by placing cotton linter samples which had undergone a varying number of wetting cycles in a common desiccator in an atmosphere of constant relative humidity. While the water vapour adsorption curve follows the same general trend as the heat of wetting curve, the results appear to be anomolous when compared to the results for nitration and methylation of the cotton linter samples.

However, all the data on cellulose can be correlated by means of the following explanation.

Dry cellulose is itself a desiccant comparable in strength to phosphoric pentoxide. Therefore, in an atmosphere of very limited relative humidity, such as is obtained in a desiccator over phosphoric pentoxide, where they were stored, the cellulose samples with the greatest accessibility will gain the most moisture. Therefore, when they are placed in an atmosphere of higher relative humidity and allowed to come to equilibrium, they will not gain as much moisture as the less accessible samples which picked up less moisture when stored over the phosphoric pentoxide. Similarly, the most accessible samples, when removed from the desiccator over the phosphoric pentoxide and placed in the calorimeter, will give smaller heats of wetting than will the least accessible samples because of the difference in initial moisture content.

Thus, it can be seen that the samples showing the greatest accessibility to the nitrating and methylating reagents, would show the least values for water vapour adsorption and heat of wetting because of the moisture (even though a very small amount) obtained while stored over the phosphoric pentoxide.

The relatively small heat of wetting obtained for the sample which had undergone no wetting cycles compared to the larger value

obtained for the sample which had undergone one wetting cycle can
be explained as follows.

To say that the sample had undergone no wetting cycles is
an arbitrary statement. It had come into contact with sodium hydroxide
solutions in the original purification process at the Hercules Powder
Plant, which would tend to swell the samples. There is also some
evidence that the benzene-alcohol mixture used to dewax the samples
will also swell them. Thus, the low value for "zero" wettings is
due to a relatively high accessibility which is offset by the zippering
effect of the first wetting cycle. Subsequent cycles then increase
the accessibility.

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